

SAFE IS NEVER SEX. IT'S DANGEROUS.



A Mario Pineda-Krch Film



THE HOT SPOT



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 ORION

The joys and perils of recombination

—

The hotspot conversion paradox and the evolution of recombination

Mario Pineda-Krch & Rosemary Redfield

SOW & D
March 20, 2003

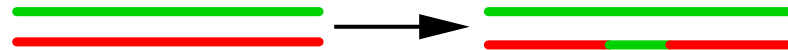
Some terminology

- *Recombination*

Aka homologous recombinational repair. **All** exchange events between homologous chromosomes that occur during meiosis.

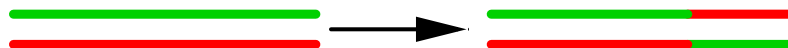
- *Conversion*

Aka gene conversion. The **non-reciprocal** transfer of genetic information from one parental chromosome to another.



- *Crossover*

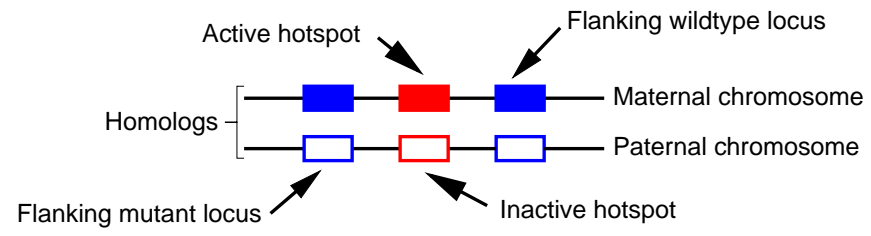
The **reciprocal** exchange of flanking markers around the branch point as the result of one type of resolution of the Holliday structure. 50% of meiotic recombinations result in crossover.



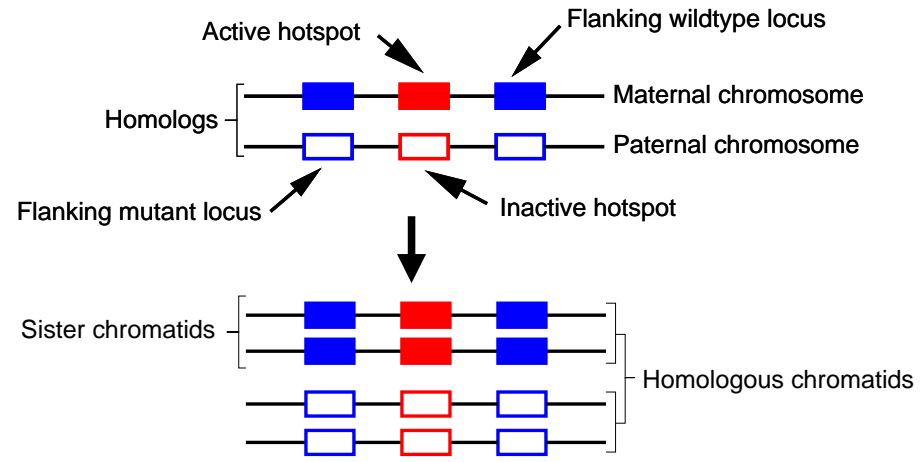
What are recombination hotspots?

- Sites on the chromosome that have a higher probability of initiating recombination than would be expected if the position of initiation would be random.
- Hotspots are defined in terms of location and an activity, e.g. sequence, genetic associations and active vs. inactive alleles (ref 5).
- Hotspots are common, ≈ 10 per chromosome in *S. cerevisiae* (ref 2)

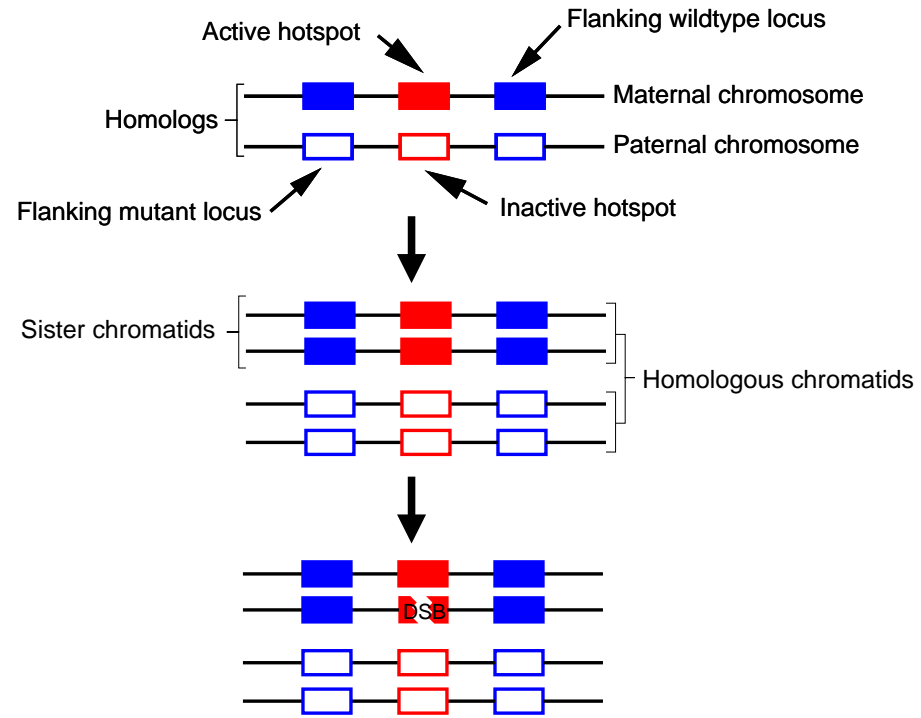
Meiotic recombination



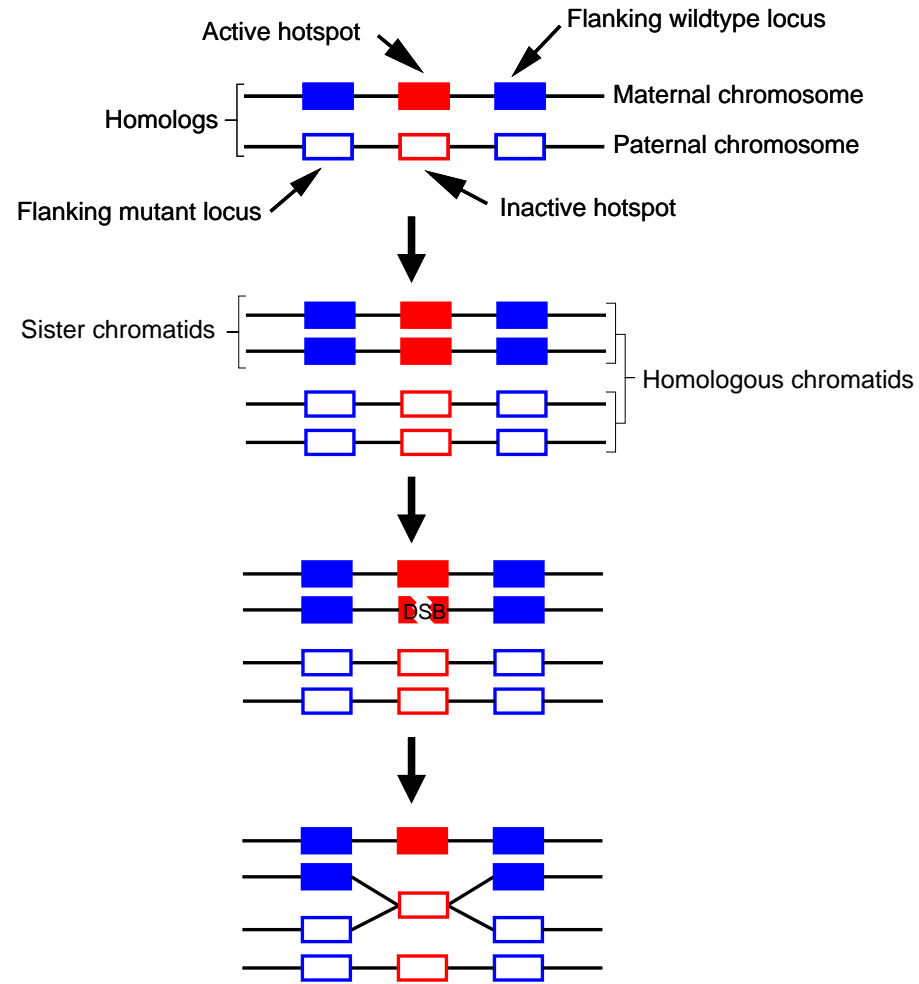
Meiotic recombination



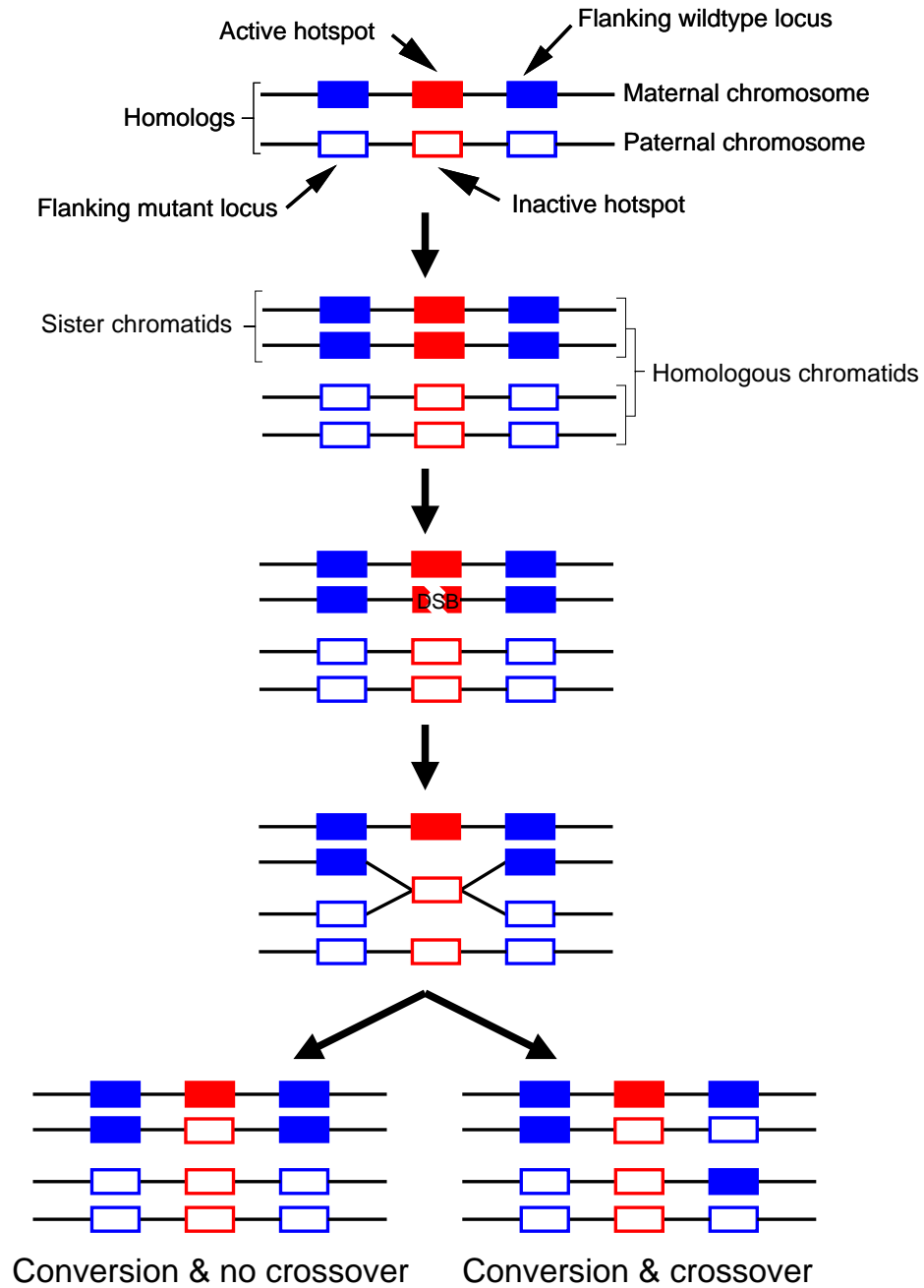
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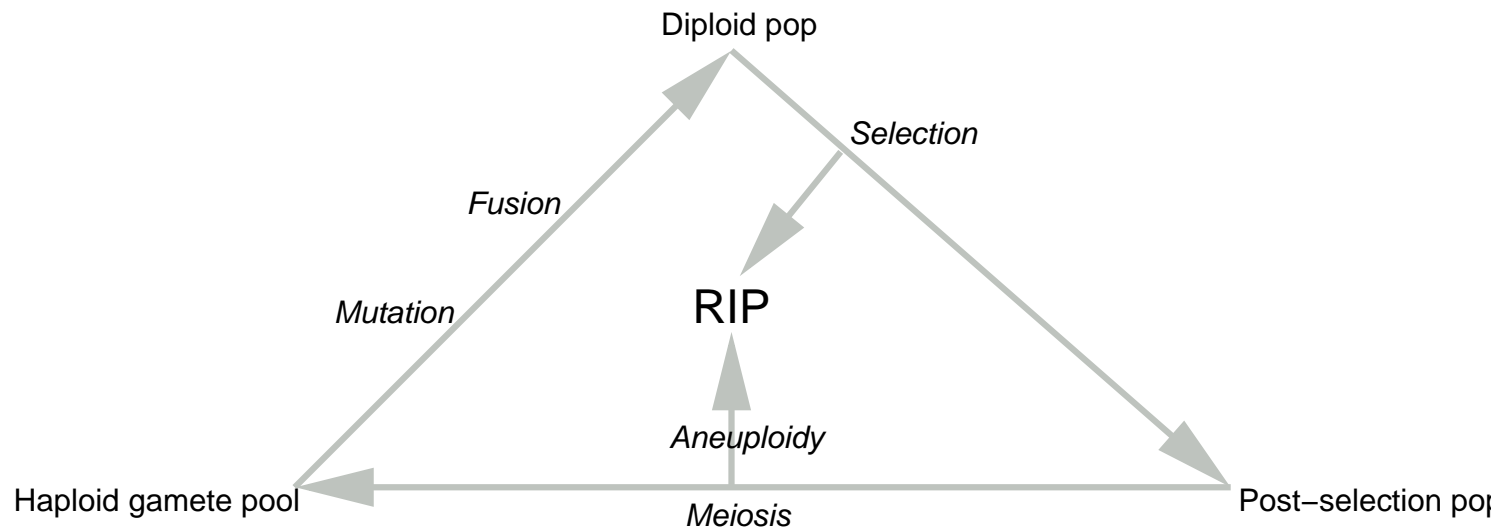
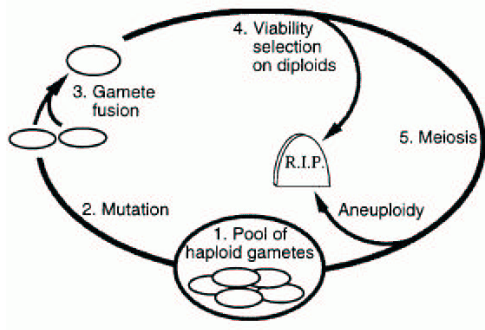
Meiotic recombination



Short and sweet...

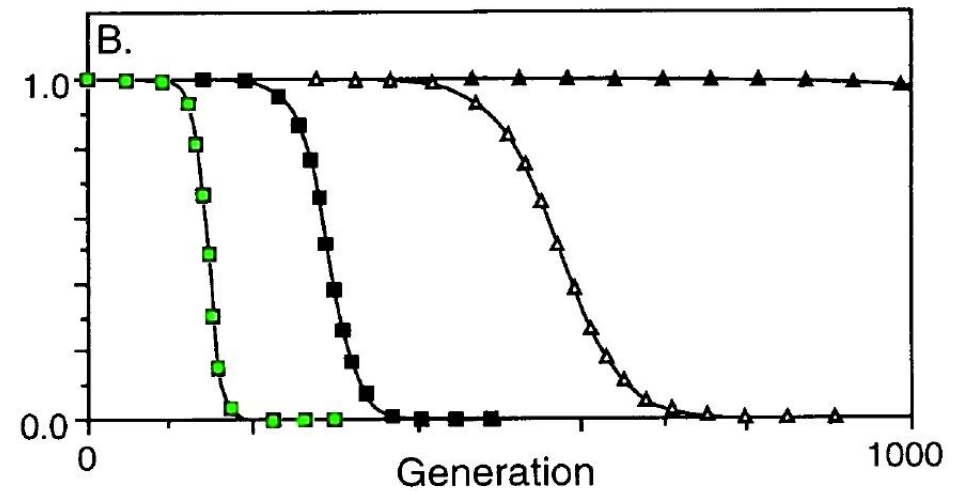
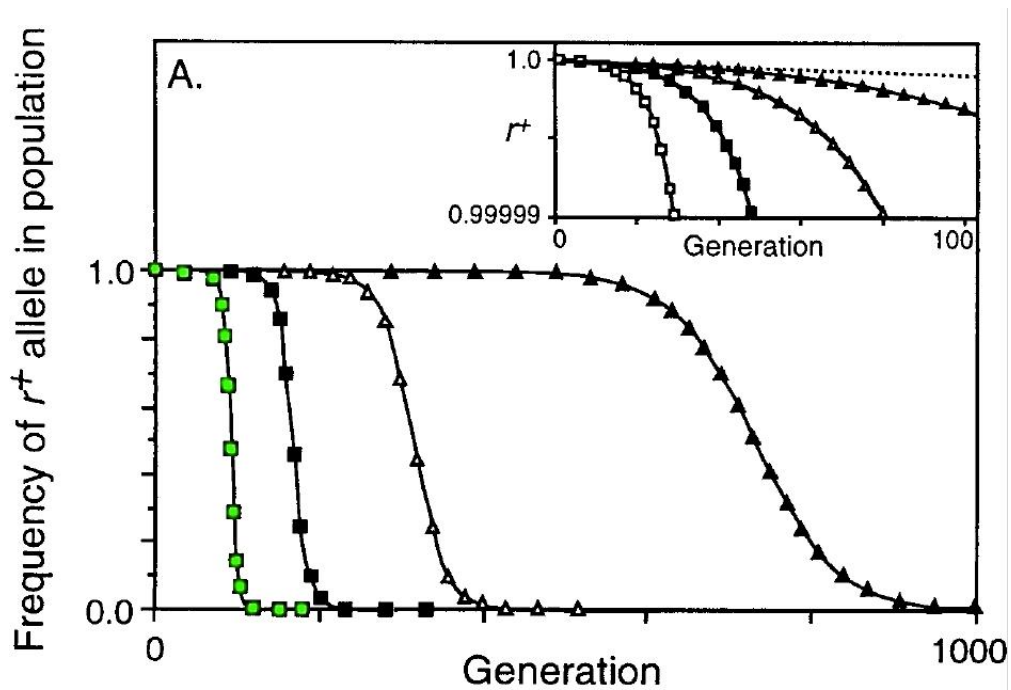
1. Recombination initiates at an active hotspot by a DSB.
2. The recombinational repair mechanism repairs the broken chromatid with a copy from its homolog (aka the template).
3. This predicts that if an inactive mutant hotspot allele arises in the population it should go to fixation. This is what the BMR paper showed! (ref 1)
4. . . . but functional hotspots remain ubiquitous!

The BMR life cycle



Some BMR results

- Prob. of initiation (aka DSB or C) $\in \{0.2, 0.1, 0.05, 0.02, 0\}$,
- $\mu_r = 10^{-8}$, $\mu_a = 10^{-1}$ ($\frac{\mu_a}{\mu_r} = 10^7!$)
- A — no selection, B — viability selection



Betty *Victor* *Carole*
GRABLE - MATURE - LANDIS



HOT SPOT

A 20th Century-Fox Picture

WITH *Laird*
CREGAR
William *Alan*
GARGAN - MOWBRAY
Allyn *Elisha*
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"SCREAMING" BY STEVE FISHER

The hotspot conversion paradox revisited

Shortcomings of the BMR model:

- single locus
- does not consider the molecular mechanisms of recombination

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We develop a multilocus model explicitly incorporating several molecular mechanisms!

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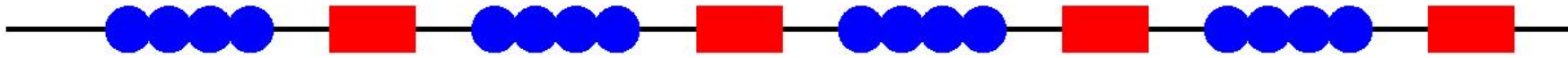
- single locus
- does not consider the molecular mechanisms of recombination

We develop a multilocus model explicitly incorporating several molecular mechanisms!

Possible results:

- **resolve:** the BMR-model was too simple
- **persists:** strengthen the paradox and provide an incentive to revisit the commonly accepted mechanism of recombination.

A multilocus framework

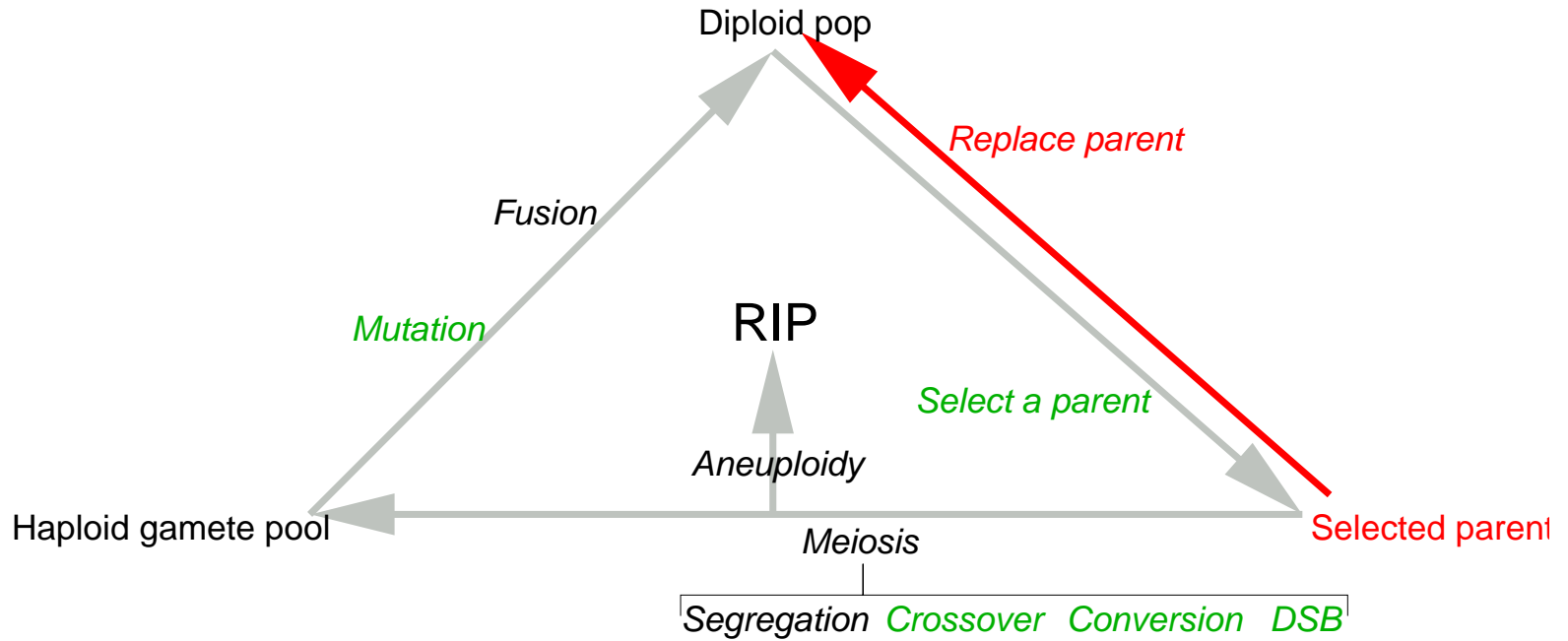
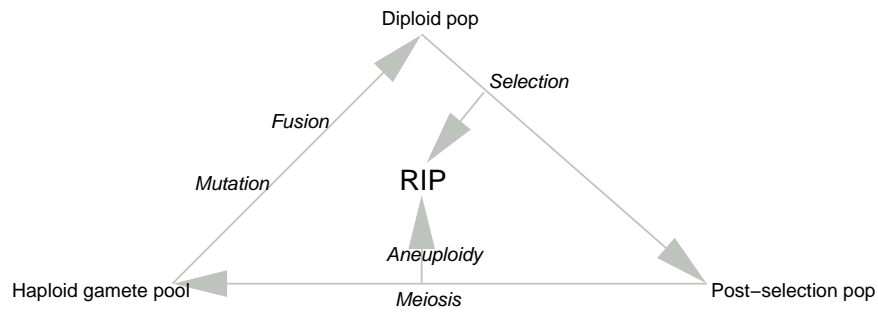


- 1 or 10 **hotspots** per chromosome
- 2 or 11 number of **flanking loci** (aka fitness loci) per chromosome
- Flanking loci (flanking regions) represent gene clusters located in-between the hotspot loci, i.e. blocks of linkage disequilibrium.
- Stochastic model using a genetic algorithm.
- Explicitly incorporating molecular mechanisms, e.g. DSB, chromatid pairing, . . .

Some assumptions

- Hotspots have a fixed location and can be defined by a sequence (aka β -type) (ref 5)
- DSB occurs **at** the active hotspot
- Hotspots have no direct effect on phenotype
- Molecular restriction incorporated
- Constant population size
- Higher mutation rate in flanking loci than on hotspot loci
- No back mutations
- Binary activity level (active / inactive)

Revised life cycle



Bears on mutation rates

An example from *S. cerevisiae* (ref 2):

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- 16 chromosomes, 5570 genes and 177 hotspots

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⇒ **flanking loci have one order of magnitude higher mutation rate than hotspot loci!**

Select a parent

1. Fitness evaluation

- The number (proportion), p , of mutant alleles at the flanking loci
- Implement the fitness function $W = (1 - s)^p$ where s is some form of selection coefficient

2. Proportional selection of a parent aka Roulette Wheel Selection

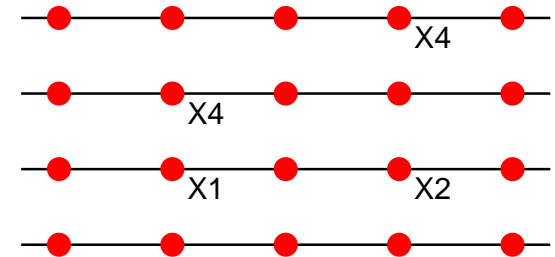
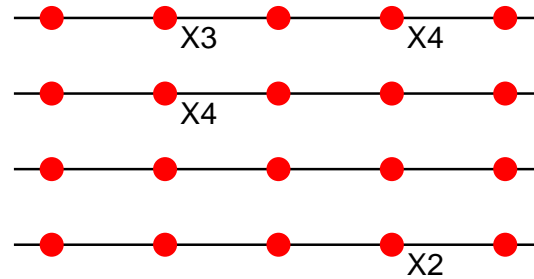
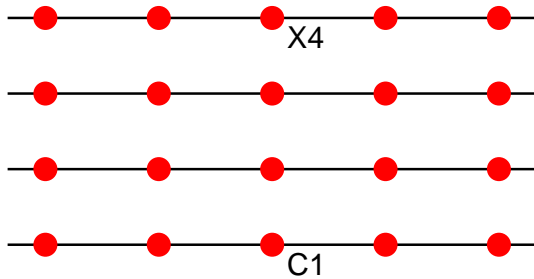
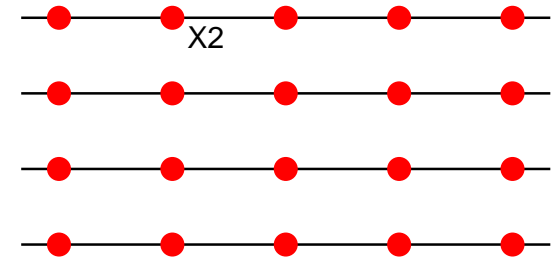
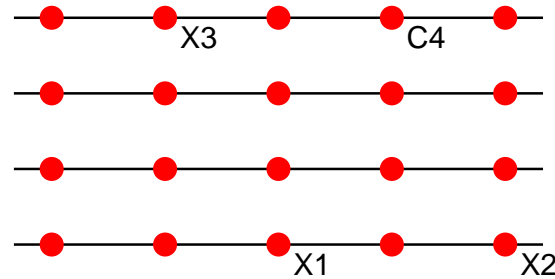
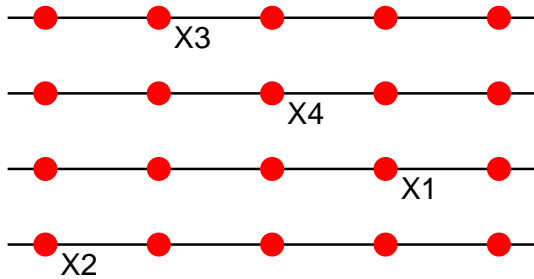
What is s ? How to count the number of mutants? Alternative fitness functions?

The perils aka the molecular mechanisms

Three are wrong, which ones?

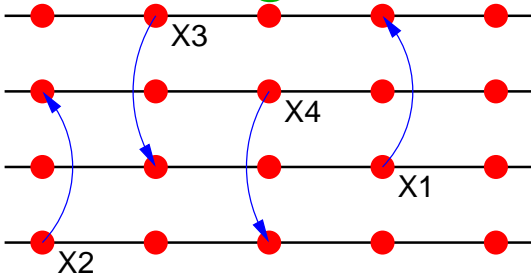
X — crossover (includes conversion), C — conversion only.

Assuming a maximum of 2 crossover participations per chromatid.

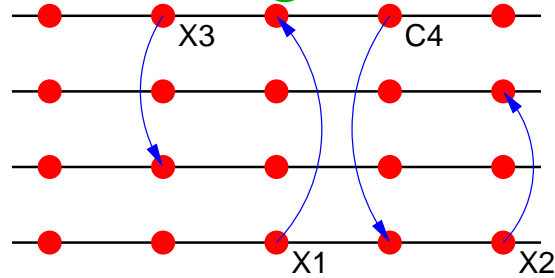


The answer

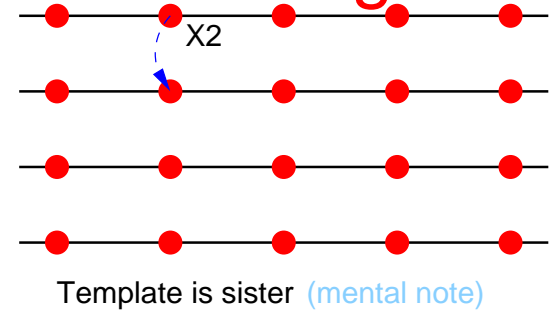
Right!



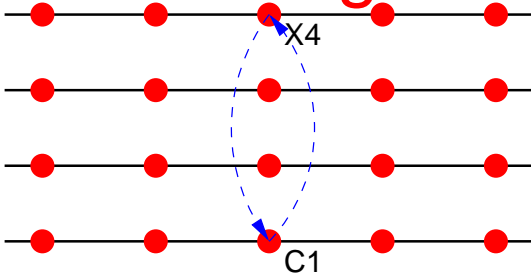
Right!



Wrong!

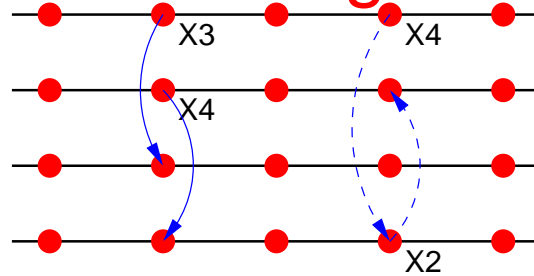


Wrong!



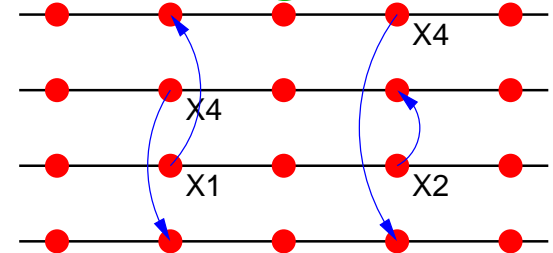
Circular template assignment

Wrong!



Left crossovers are correct
Right crossovers wrong; template broken

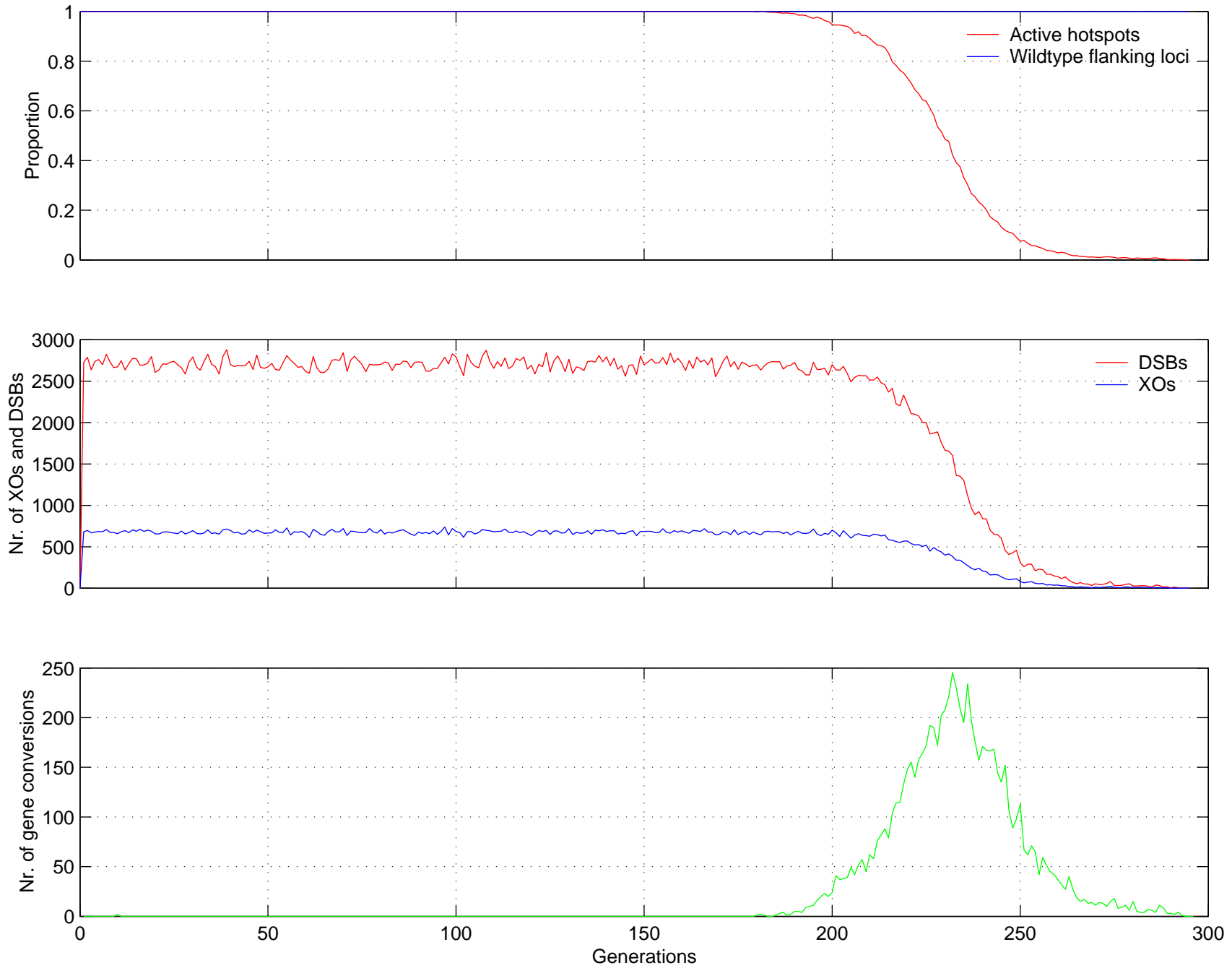
Right!



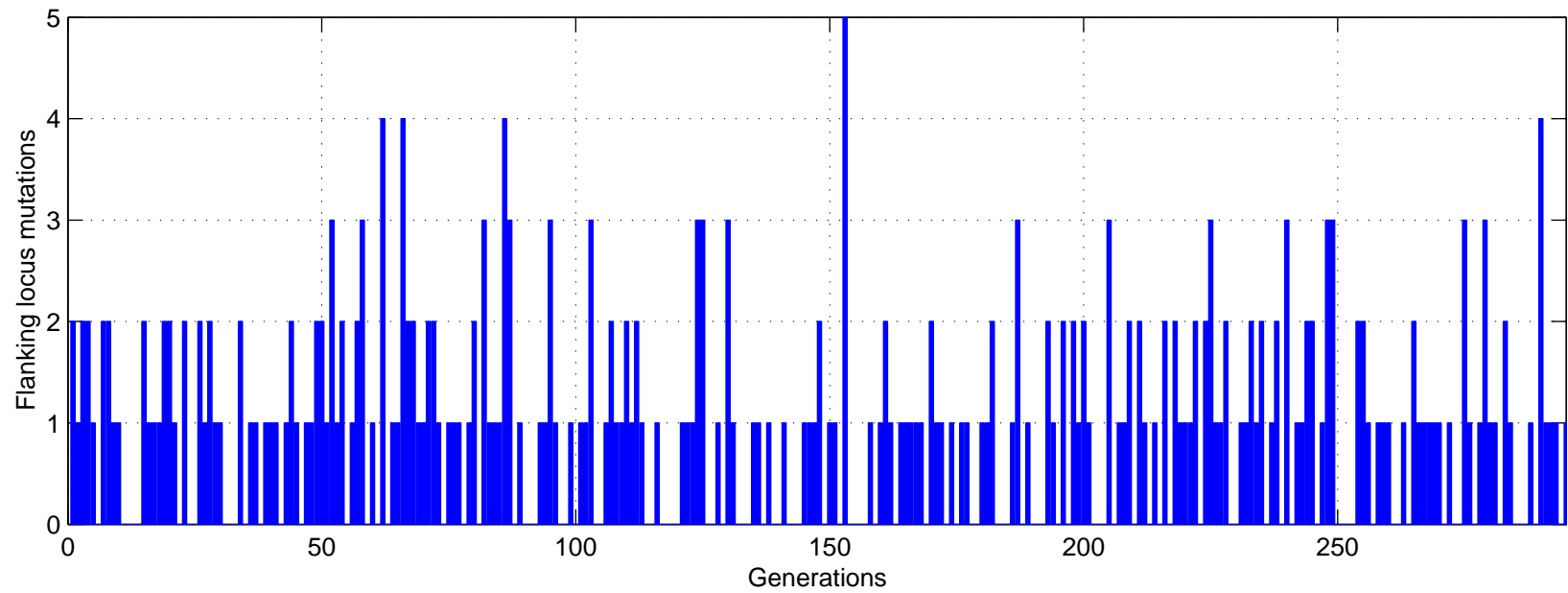
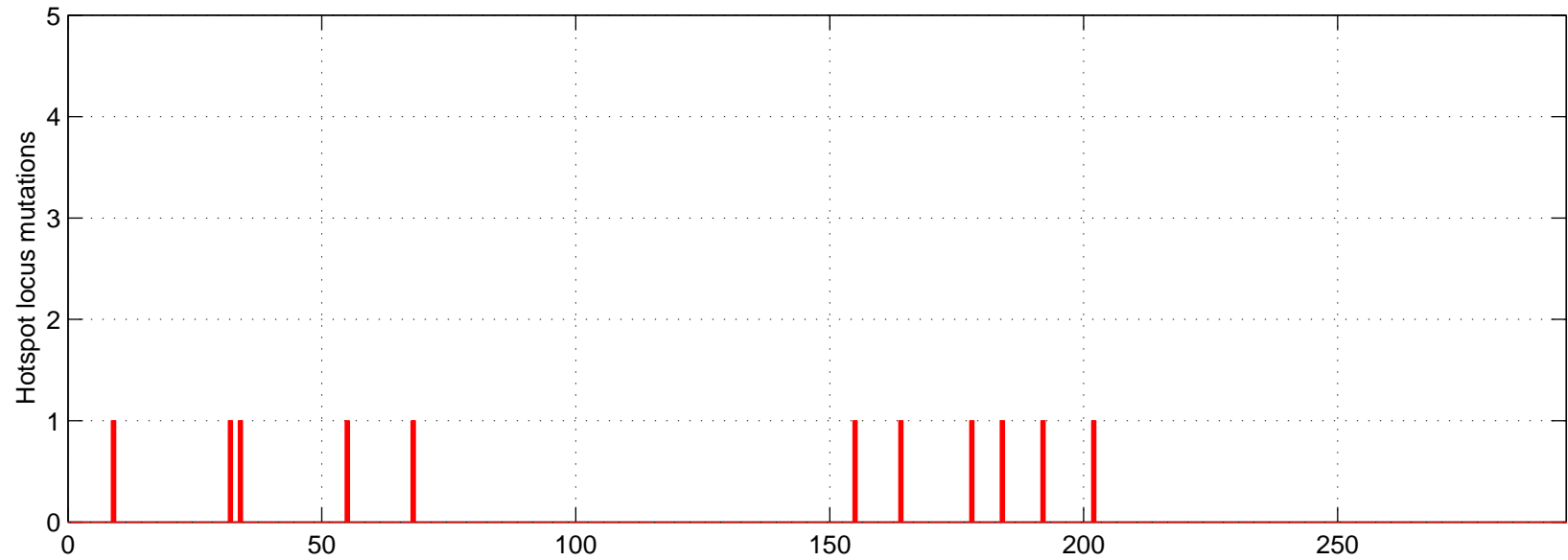
Some model parameters

Parameter	Definition	Typical value
N	Nr. of chromosomes in the population	$N = 10000$
n_r	Nr. of hotspot (r -loci) loci per chromosome	$n_r = 1$ or 10
C	Prob. of DSB per active hotspot aka temperature	$C = 0.2$
μ_a	Per generation mutation prob. of a flanking locus	$\mu_a = 10^{-4}$
μ_r	Per generation mutation prob. of a hotspot locus	$\mu_r = 10^{-5}$
s	Selection coefficient	$s = 0.7$
n_x	Maximum number of crossover initiations per chromatid	$n_x = 2$
X	Crossover probability at a DSB	$X = 0.5$

Single locus, $N=5000$, $\eta_r=1$, $C=0.2$, $\mu_a=10^{-4}$, $\mu_r=10^{-5}$, $s=0.7$, $\eta_x=2$ (HS2003-3-20-9-17-21)



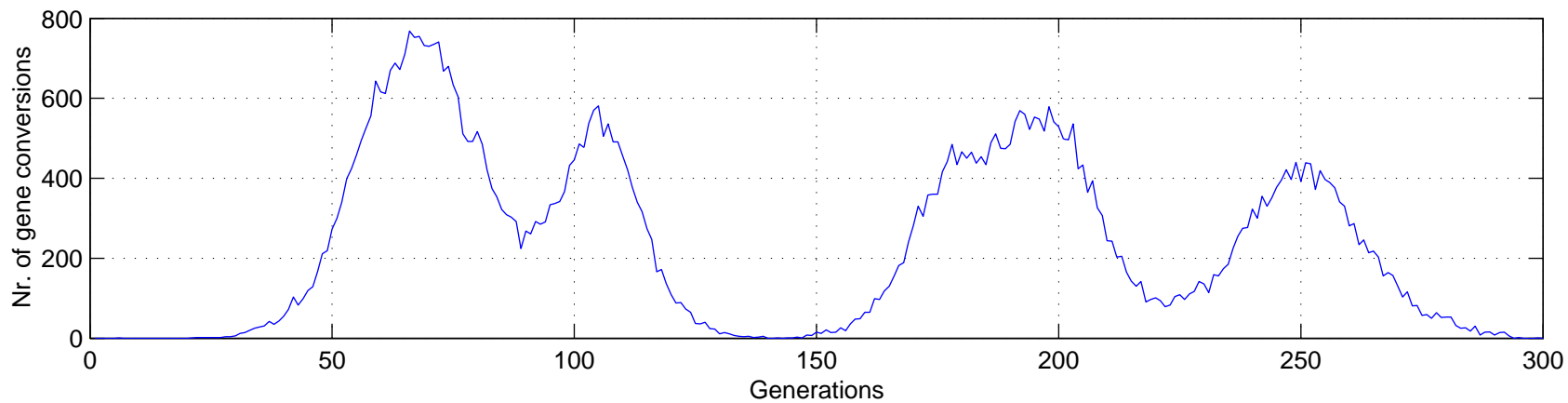
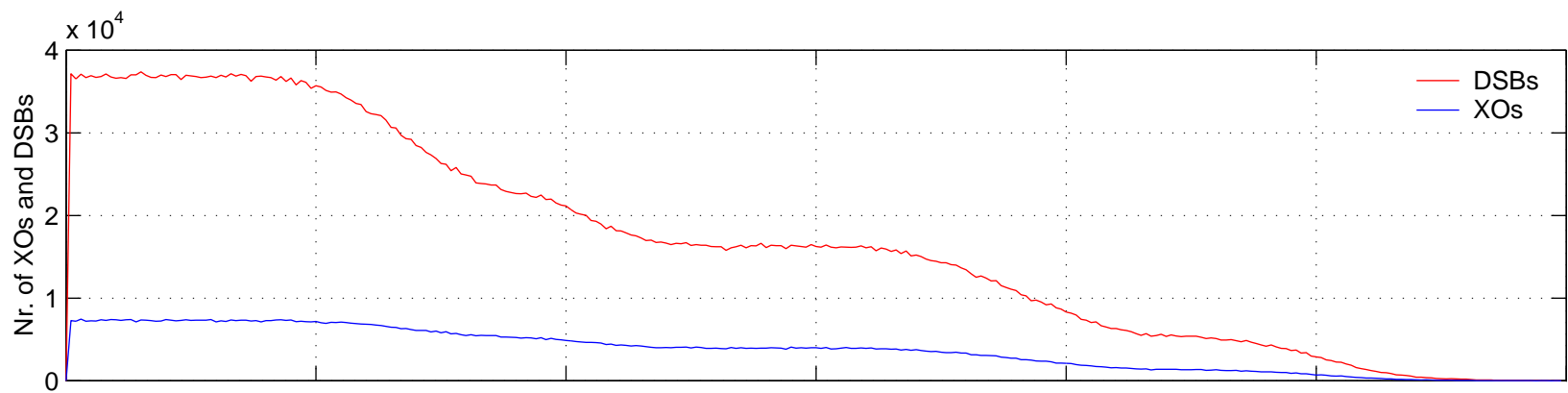
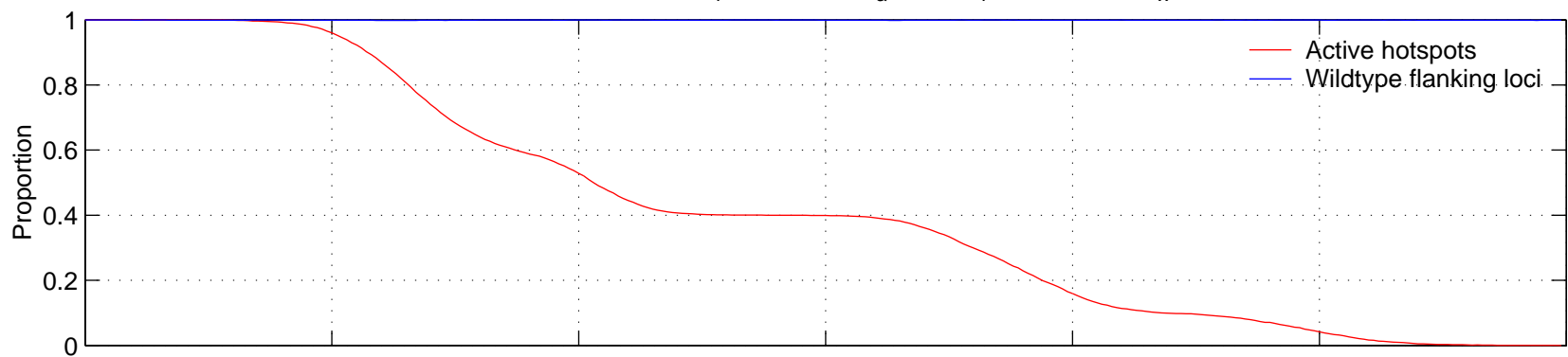
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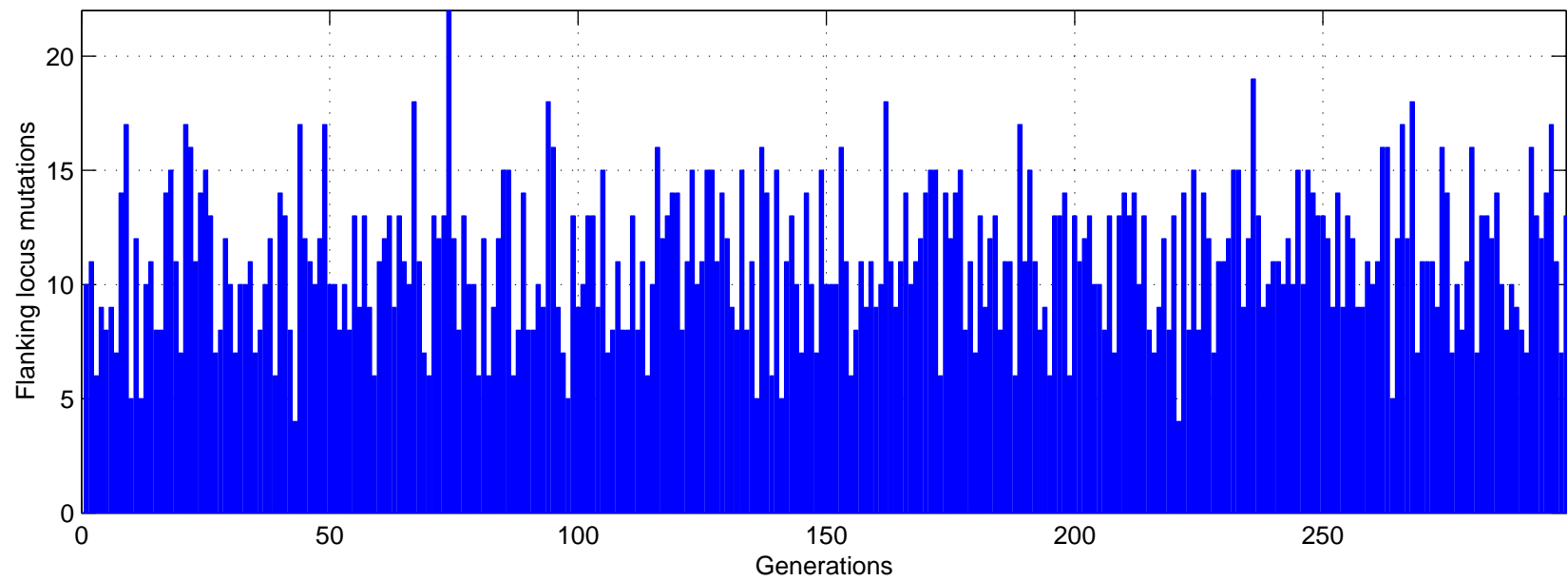
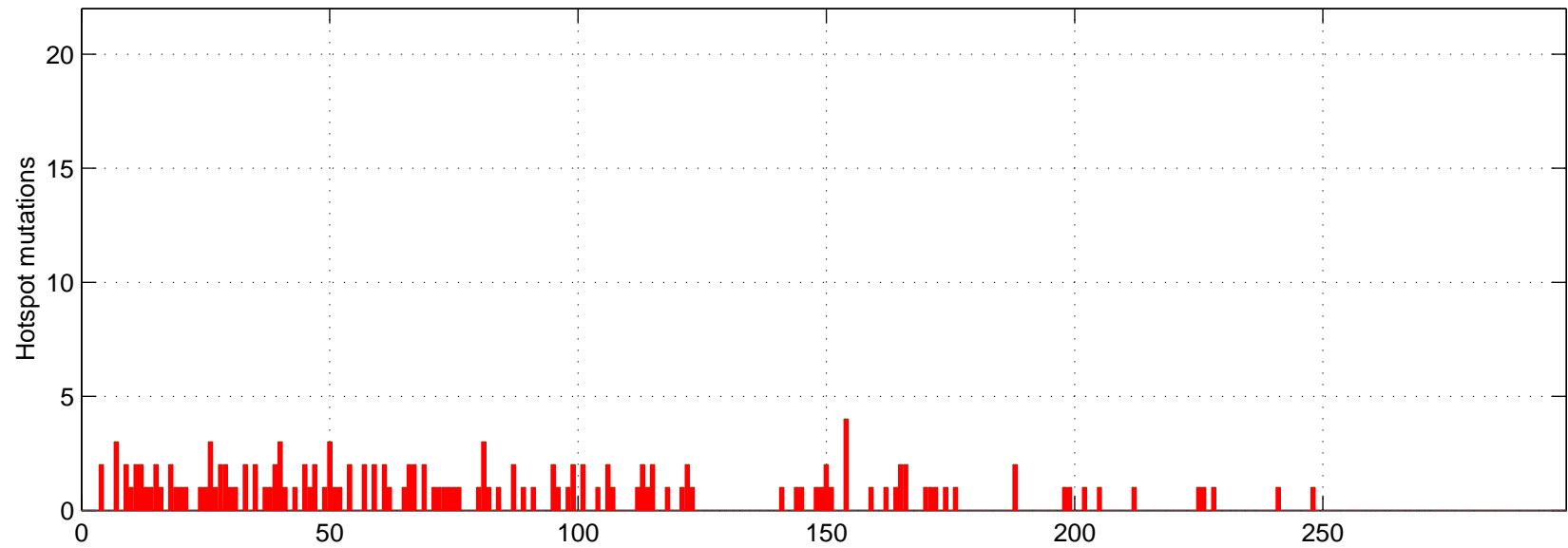
The stochastic model vs the probabilistic model (i.e. BMR)

- The paradox persists!
- The demise takes longer (≈ 300 gen. vs ≈ 100 gen.)
- Lag period prior to the start of the conversion
- Once the demise starts, it is just as fast (≈ 100 gen.)

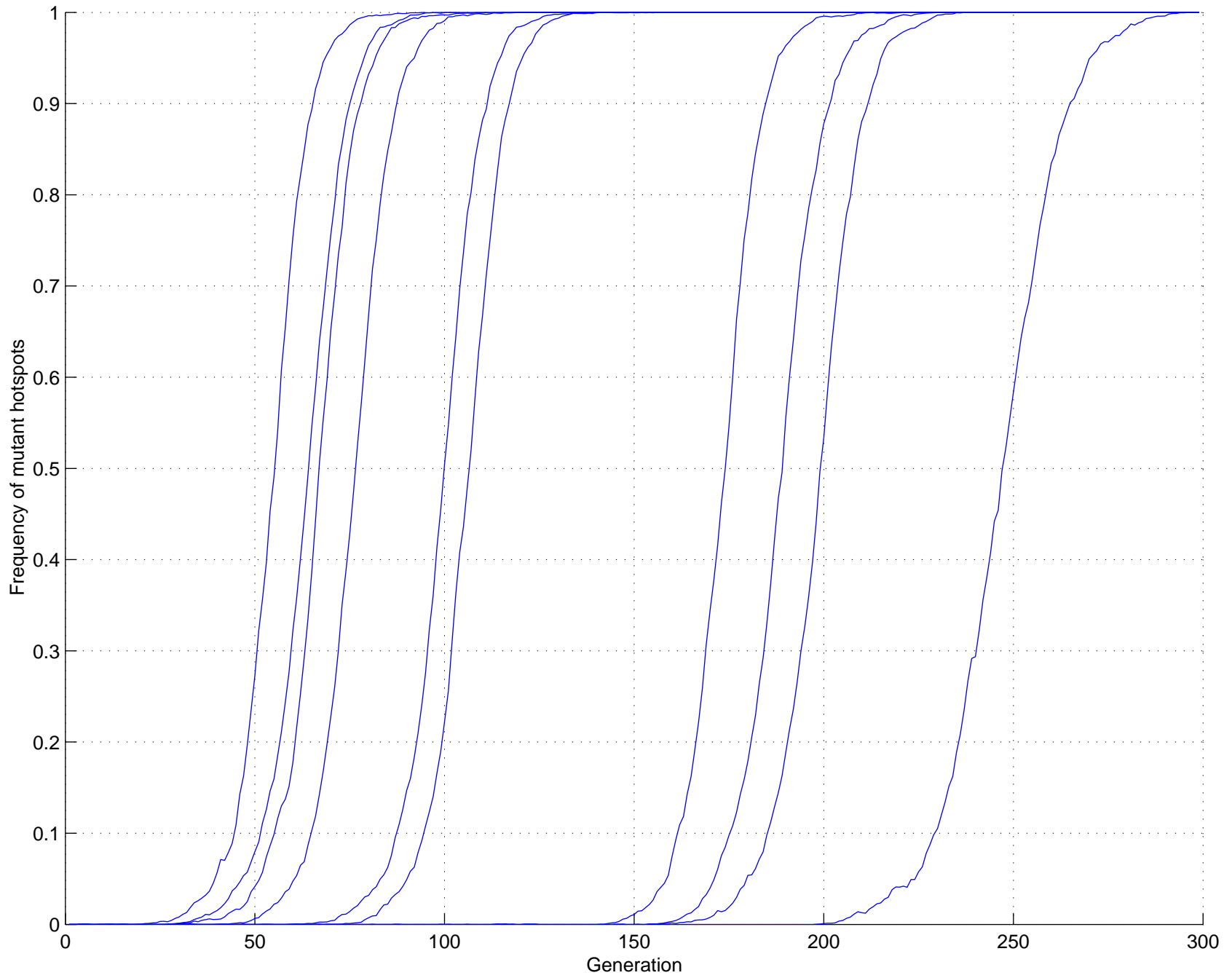
Multilocus, $N=10000$, $n_r=10$, $C=0.2$, $\mu_a=10^{-4}$, $\mu_r=10^{-5}$, $s=0.7$, $n_x=2$



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Multilocus, $N=10000$, $n_r=10$, $C=0.2$, $\mu_a=10^{-4}$, $\mu_r=10^{-5}$, $s=0.7$, $n_x=2$



Multilocus vs single locus

- The paradox still persists!
- Extinction takes longer
- Shorter lag period before decline starts
- The fixation of inactive alleles is driven by a conversion ratchet.
- The ratchet does **not** gain momentum due to mutation accumulation
- The time lag between subsequent conversions seems increases as the number of active hotspot loci decrease.

⇒ **mutation seeds & conversion drives!**

How are active hotspots maintained?

- Are the current ideas of the molecular mechanisms of recombination flawed or incomplete?
- Counteracting forces?
- (Re)generation of active hotspot?

Problems:

- what makes a hotspot?
- what is the dynamics of hotspots?

Modifying the assumptions

- Hotspots have a fixed location and can be defined by a sequence (aka β -type) (ref 5)
- DSB occurs at the active hotspot \Rightarrow Asymmetric exchange, e.g. crossover asymmetry (ref 3)
- Hotspots have no direct effect on phenotype \Rightarrow Hotspots having non-meiotic benefits (NMB).
- Molecular restriction incorporated \Rightarrow Allow sister chromatids as templates (20%)
- Constant population size
- Higher mutation rate in flanking loci than on hotspot loci
- No back mutations
- Binary activity level (active / inactive) \Rightarrow A continuum of hotspot activity. (ref 4)

“The factors responsible for variation in recombination remain obscure,”
— Nachmann 2002

“. . . the nature of recombination prone regions remain obscure.”
— Svetlova et al. 2001

“. . . the paradox . . . is likely to be resolved only by significant changes to the commonly accepted mechanism of crossing over”
— Boulton et al. 1997

“If the facts don’t fit the theory, change the facts.”
— Albert Einstein

Thanks!

Sally & Lab, Jeremy Barbay, IAM

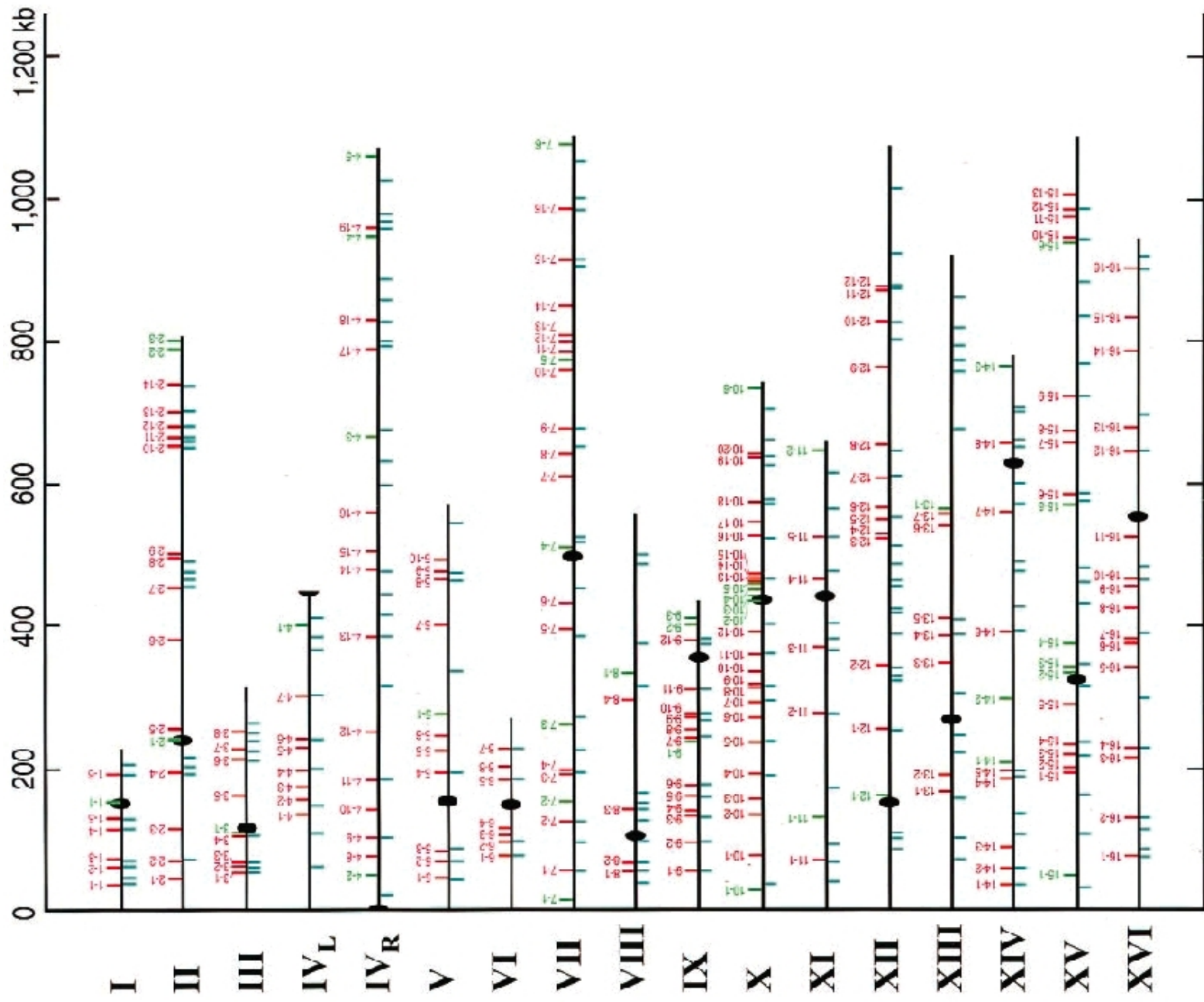
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- [2] J.L. Gerton, J. DeRisi, R. Shroff, M. Lichten, P.O. Brown, and T.D. Petes. Global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences USA*, 97(21):11383–11390, 2000.
- [3] A.J. Jeffreys and R. Neumann. Reciprocal crossover asymmetry and meiotic drive in a human recombination hot spot. *Nature Genetics*, 2002.
- [4] M.W. Nachmann. Variation in recombination rate across the genome: evidence and implications. *Current Opinions in Genetics & Development*, 12:657–663, 2002.
- [5] T.D. Petes. Meiotic recombination hot spots and cold spots. *Nature Genetics*, 2:360–369, 2001.

Appendix

Hotspot stats for *S. cerevisiae*

- hotspots are associated with regions of high G + C base composition
- coldspots are associated with centromeres and telomeres
- in theory there are different types of hotspots:
 - α require transcription factor binding, not sequence specific
 - β do not require binding of TF, is sequence specific
 - γ associated with G + C peaks
- ... but we cannot distinguish between the types!
- 177 hs' identified in *S.c.*
- Average number of hs per chromosome: 11
- 56% of hs are associated with G + C peaks



The concept of a genetic algorithm

- GAs use operators derived from genetics (e.g. mutation, selection, recombination, fitness, . . .) to evolution of a set of strings.
- Each chromosome is a string encoding states or values.
- Selection depends on some rating of the strings (fitness) based on the encoded values.
- Strings are copied (reproduce) or removed (selective death) in each generation
- The copied strings (survivors) are then modified by various genetic operations (e.g. mutation and recombination)
- The modified strings are inserted into the population, replacing other strings, so that the population size is constant.